Plankton analysis by automated submersible imaging flow cytometry: Transforming a specialized research instrument into a broadly accessible tool and extending its target size range

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LONG TERM GOALS

Detailed knowledge of the composition and characteristics of the particles suspended in seawater is crucial to an understanding of the biology, optics and geochemistry of the oceans. The composition and size distribution of the phytoplankton community, for example, help determine the flow of carbon and nutrients through an ecosystem and can be important indicators of how coastal environments respond to anthropogenic disturbances such as nutrient loading and pollution. Our goal is to provide researchers with instruments to continuously monitor phytoplankton community structure and investigate questions about the world's ocean ecosystems.

OBJECTIVES

Flow cytometry is one of the most promising technologies for studies of the microscopic constituents of marine ecosystems (Moore et al. 2009; Sosik et al. 2009). The intent of this project is twofold: to commercialize a field-proven state-of-the-art submersible imaging flow cytometer for nano- and microplankton so that other researchers can utilize this exciting new technology, and to develop a next generation of the instrument with extended measurement range, capable of analyzing cells from pico- to microplankton.

APPROACH

We are developing a prototype commercial version of Imaging FlowCytobot (IFCB), reproducing its functions via a series of modular components whose integration will result in a simple and robust instrument that is both reliable and easy to manufacture. The first step involved a ground-up examination of an existing benchtop version of Imaging FlowCytobot. This examination established design goals for each functional module of the instrument (e.g., flow system, cell detector, imaging system, signal processing electronics, control system). The redesign process began with a mechanical backbone analogous to the optical breadboard now used, onto which have been designed core functional modules for cell detection and imaging, to establish a working imaging system that utilizes electronics and fluidics similar to those in the present Imaging FlowCytobot. This approach will

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enable us to compare performance of the commercial prototype to that of the original instrument. Problems with components or integration (such as incorrect physical layout or optical components) will be corrected, followed by redesign and fabrication of new components. Image quality of this core system has been shown to be satisfactory and we are continuing with redesign and evaluation of the other aspects of the instrument. The upgraded benchtop unit is now being tested in the laboratory, and we are constructing a new pressure housing for it in order to conduct field tests. In collaboration with University of Washington (E. Armbrust's laboratory), we are also investigating approaches to efficiently obtain both large- and small-dimension laser spots (dual beam), for simultaneous detection of pico- and microphytoplankton, using a newly-developed position sensitive detector.

WORK COMPLETED

Target goals for system improvement have been identified along with methods for achieving payload, power, and cost savings. We have been investigating ways to reduce size and power requirements, and increase duration, as described below.

Opto-Mechanics. We designed and fabricated a prototype of a more rigid opto-mechanical system that we expect will be more stable and easier to align. We are in the process of evaluating its performance. The new design includes compact mounts for the flow cell (Fig. 1), the laser (Fig. 2), and the PMT block (Fig. 3). In the course of discussions about the optical layout, we realized that it might be possible to increase the resolution of images (by using an objective with higher numerical aperture and a flow cell that produces a thinner sample core stream), so we investigated this possibility. We concluded that it is possible but not practicable for the present instrument because the decreased depth of focus would necessitate reducing the sample throughput rate and using a larger format camera.

Syringe pump. The commercially-available syringe pump system used in the original IFCB requires 22W. We have designed and fabricated a new pump with much smaller motors that require only 3W. The new pump also has the drive mechanism vertically aligned for compactness (Table 1; Fig. 4).

Flash Illumination. The xenon flash lamp in the original IFCB is relatively large, with a separate power supply. It also requires a custom modification to reduce its flash duration to the 1-microsecond level needed for blur-free images; this modification requires a custom-fabricated (and bulky) electromagnetic shield, which in turn forces the light source to be located far from the condenser, with a fiber optic bundle delivery system. We have replaced this system with a newly-available module (from Hamamatsu) with integrated power supply and shielding, which is small enough to locate directly at the condenser input of the optical system. Besides being much smaller than the original, this system requires less power, is less expensive, and does not require modification to attain short-duration flashes (Table 1). The new flash lamp has different light output geometry, but we were able to redesign the condenser optics to obtain images with quality comparable to those of the original.

Computer. We are using a new computer based on an Atom processor, which uses only 5 W of power (as compared to 18W for the computer in the previous instrument; Table 1).

Long-duration syringe. In response to our request, the syringe manufacturer (Kloehn, Inc.) has developed a syringe with tighter tolerances for improved cold-water performance (and presumably longer lifetime). This should eliminate the need to manually select syringes. We are currently testing the first of these syringes in the original instrument.

Backup sheath pump. The motors in our current micro gear pumps (which we selected because of their low power requirement) have a design lifetime of ~1 yr (though some have lasted much longer). Since these are relatively expensive, we prefer not to replace them for every deployment, so we have now installed 2 pumps in parallel, under computer control. This allows us to run a motor until it fails, but then continue on the backup pump for the remainder of a deployment. This has already enabled 2 extended deployments (the record for longest deployment of IFCB is now nearly a year).

GigE camera. We have switched to a new kind of camera (Prosilica 1380H), which uses the same CCD chip as our original camera (and therefore produces the same quality images) but is smaller and does not require a frame grabber board. This reduces both size and cost.

Software. We are working with Martin Cooper Consulting to update the software for data acquisition and analysis. The most significant advance on this front is that we have implemented a custom method for capturing the "region of interest" in each image in real time, which will allow us to avoid using expensive commercial software ($3000 plus $400 license fee per instrument) for this purpose.

Housing. The present prototype (Fig. 5) will fit inside a 7.4 inch diameter housing, which will reduce the size and weight of the instrument by several fold (Table 1).

Commercial partner. Our objectives require a commercial partner to produce and market the new IFCB design. The partner we originally identified was acquired by a larger company and is no longer able to work with us in the way we envisioned. To address this challenge, we have begun new discussions with McLane Research Laboratories, Inc. We have signed non-disclosure agreements and met twice with both management and engineers. We plan to work together this fall to finish assembly of the first beta unit and define a mutually agreeable path forward that will likely include a license agreement.

RESULTS

We have produced a new prototype incorporating a more rigid optical structure. We have produced a new prototype syringe pump/distribution valve system and are using a new energy efficient computer and camera. These changes combine to reduce the instrument’s power requirement significantly, which will enhance its utility for non-cabled platforms. We have also obtained improved syringes and implemented and tested a dual gear pump system that will allow longer deployments, increase reliability, and reduce maintenance costs.

IMPACT/APPLICATIONS

National Security

There is potential for this application to be useful for detecting pathogens in water supplies.

Economic Development

The Imaging FlowCytobot represents a potential new product line, since it has utility for plankton ecologists studying plankton processes (including effects of pollution and climate and change), and also for water resource managers (as a means to monitor harmful algal species).

Quality of Life

Species-level information is critical for such societally important problems as understanding the regulation and fate of regional harmful algal blooms. At the global scale, it is becoming increasingly evident that simple nutrient-phytoplankton-zooplankton models are inadequate for predicting effects of environmental change and that biogeochemical functional groups such as nitrogen fixers, silicifiers, and calcifiers need be resolved. We presently lack observational capabilities to provide data for building and evaluating models, as well as for developing new approaches such as satellite-based remote sensing approaches to monitor functional group distributions. Widespread availability of instruments such as Imaging FlowCytobot will be an important step to overcoming present observational limitations.

Science Education and Communication

The images of individual plankton cells provided by these instruments, remotely and in near-real time, should contribute effective components of educational programs about the oceans, both in science curricula and for the general public.

TRANSITIONS

Quality of Life

A prototype Imaging FlowCytobot has already provided early warning of a toxic dinoflagellate blooms in the Gulf of Mexico (the first toxic *Dinophysis* bloom observed in Texas waters, and subsequent *Karenia brevis* blooms), allowing timely closure of shellfisheries that prevented human illnesses.

Science Education and Communication

Images from a prototype Imaging FlowCytobot have been circulated to plankton experts via the Internet, allowing species identification and better interpretation of potential processes behind bloom dynamics.

RELATED PROJECTS

This project builds on previous projects in the Olson and Sosik laboratories See http://www.whoi.edu/sites/hsosik/ for more details.

PUBLICATIONS

Moore, C., A. Barnard, P. Fietzek, M. R. Lewis, H. M. Sosik, S. White, and O. Zielinski. Optical tools for ocean monitoring and research. 2009. Ocean Science. 5, 661–684.

Sosik, H. M. 2008. Characterizing seawater constituents from optical properties. In M. Babin, C. S. Roesler and J. J. Cullen [eds.], Real-time coastal observing systems for ecosystem dynamics and harmful algal blooms: Theory, instrumentation and modelling. UNESCO, p. 281-329.

Sosik, H. M., R. J. Olson, and E. V. Armbrust. 2010. Flow cytometry in plankton research. *In* D. J. Suggett, O. Prasil and M. A. Borowitzka [eds.], Chlorophyll a fluorescence in aquatic sciences: methods and applications. Springer.

Table 1. Summary of improvements to date for IFCB design

Component	Research Prototype	Commercial Prototype
Housing diameter	33 cm	20 cm
Weight (est.)	77 kg	28 kg
Syringe pump	22 W, 15 cm deep	3 W, 9 cm deep
Computer	18 W, 400 cc	5 W, 200 cc
Flash lamp	1.5 W (est.), 960 cc	0.15 W (est.), 193 cc
Control electronics	3 W (est.), 1375 cc	1 W (est.), 230 cc
Optics	13 movable parts	4 movable parts
Camera	164 cc Frame grabber required	77 cc No frame grabber
Laser mount	5 movable parts 14 cm depth	3 movable parts 8 cm depth

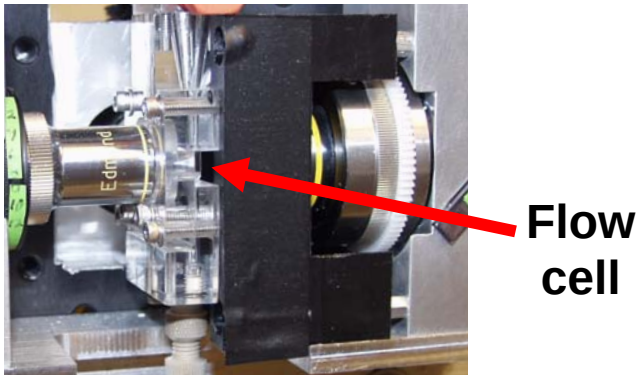

Fig. 1. New flow mount is integrated into the optical block, facilitating alignment.

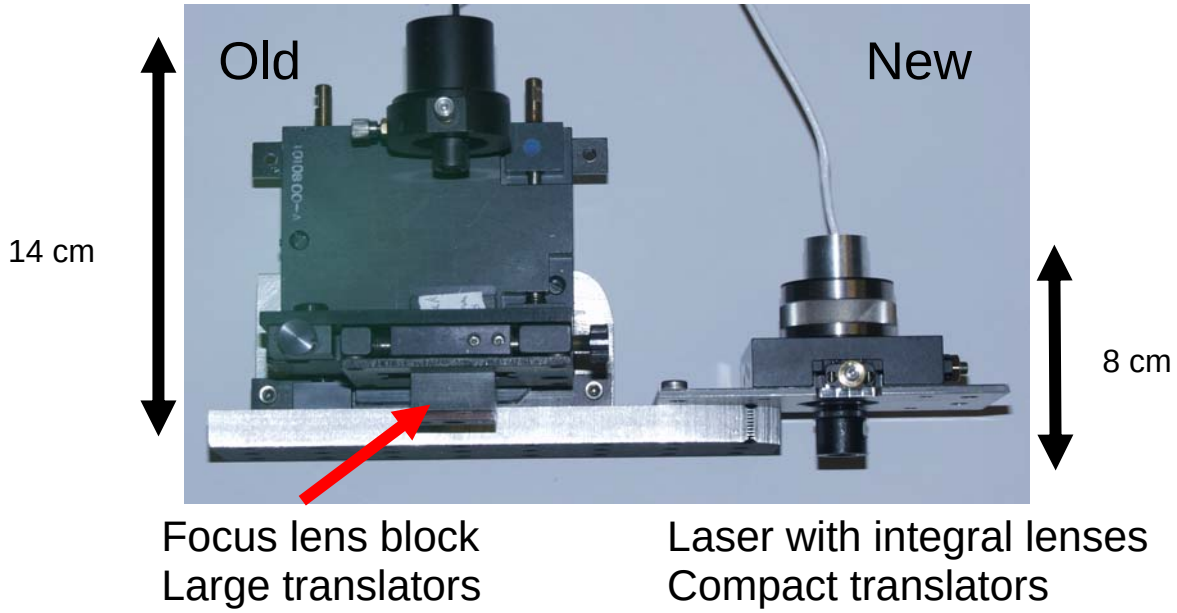

Fig. 2. Comparison of old and newlaser mounts, emphasizing reduction in size and degrees of freedom for adjustment.

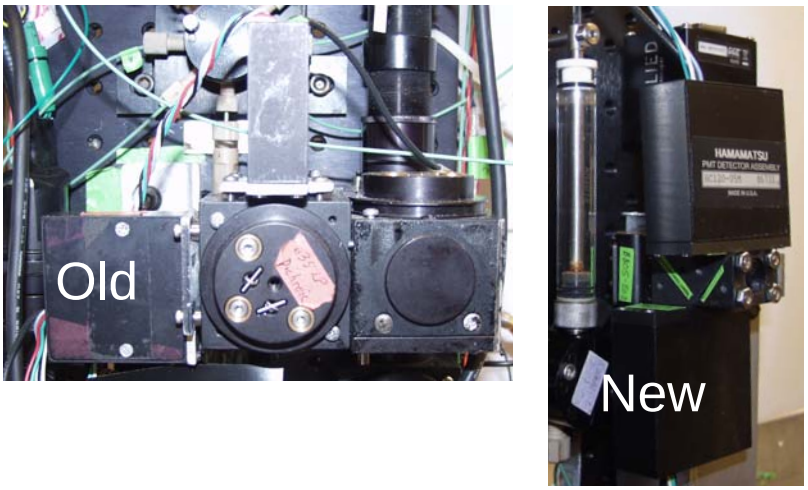

Fig. 3. Comparison of old and new PMT blocks, emphasizing reduction in size and degrees of freedom for adjustment.

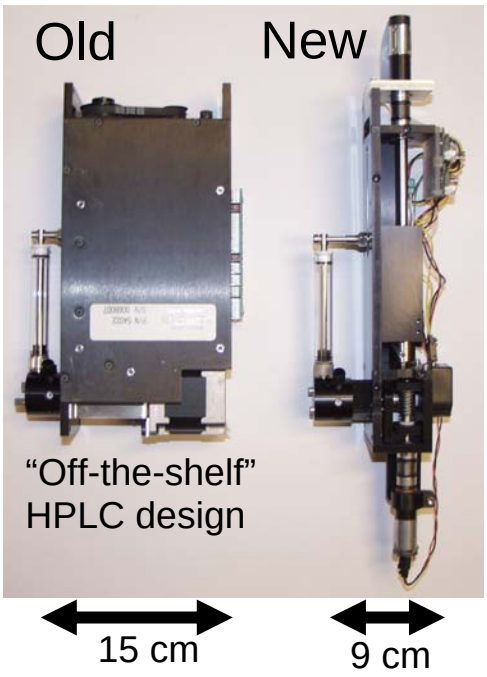

Fig. 4. Comparison of old and new syringe pump designs emphasizing reduction in horizontal dimension.

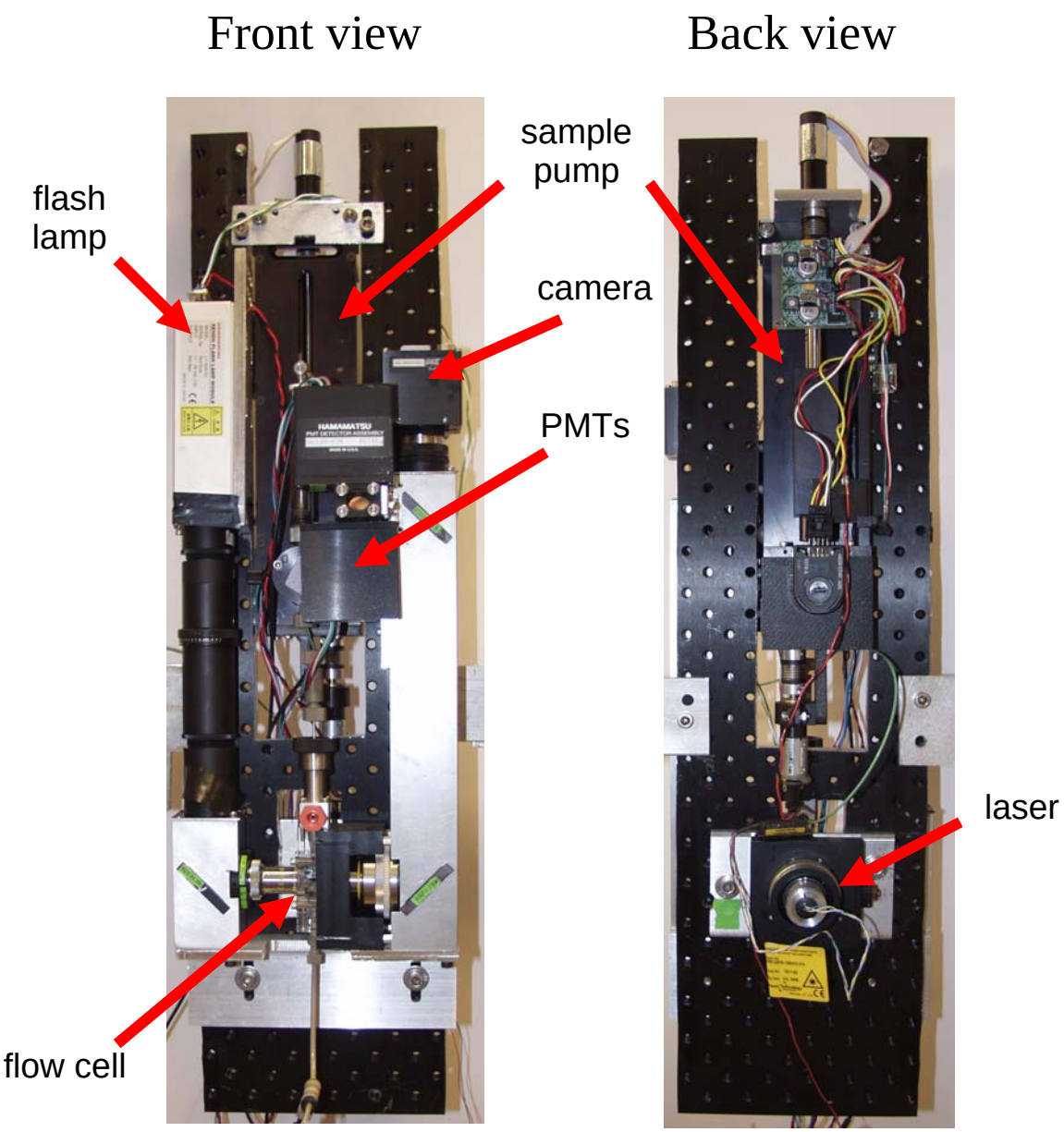

Fig. 5. Nearly complete assembly of first beta unit awaiting installation of control electronics and additional fluidics (sheath and reagents).